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Daniel Amber Shull

ENTITLED Variation of Heterogeneous Rate Constant with pH of Unbound

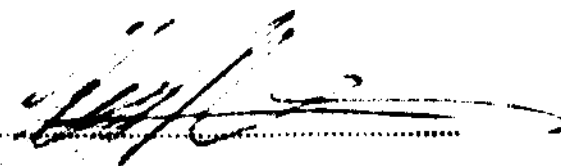
Mamallian Cytochrome c as Determined by Cyclic Voltammetry

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Bachelor of Science

Instructor in Charge

APPROVED:



HEAD OF DEPARTMENT OF Chemistry

Variation of Heterogeneous Rate Constant with pH
of Unbound, Mamallian Cytcchrome c
as Determined by Cyclic Voltammetry

By

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Thesis

for the
Degree of Bachelor of Science
in Chemistry

College of Liberal Arts and Sciences

University of Illinois

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INTRODUCTION

Mitochondria are the power plants of all living cells, providing energy the cell requires in order to carry on every day life processes. They produce energy as a product of respiration, the chemical process in which a cell absorbs oxygen and gives off carbon dioxide. Mitochondrial respiration involves four macromolecular complexes that form a chain, along which electrons are transported by two smaller "shuttles", coenzyme Q and cytochrome c [1]. As shown in Figures 1 and 2, cytochrome c transports electrons from complex III to complex IV. How the protein does this mechanistically continues even today to be a subject of intense interest.

Concepts such as binding sites, inner or outer sphere electron transfer, even quantum mechanical tunneling have all been suggested in theory, yet no single model has experimentally been shown to be definitive. In general

these models all seek to address questions of dynamics (or the lack thereof) - whether or not cytochrome c migrates, pivots, or merely rests motionlessly between the two complexes [2].

One possible way to elicit information about electron transfer of free, mammalian cytochrome c might be to vary the external pH. In theory, this could alter the asymmetric charge distribution on the surface of the molecule, thus changing the orientation of the dipole, and perhaps the conformation of the protein at an interface at which charge transfer might occur. Such changes, if significant enough in their contribution to the overall process of electron transfer, should manifest themselves in experimental parameters such as the rate constant for heterogeneous charge transfer at an electrode, an electrochemical measure of the reversibility of a redox system [3]. If the results of such preliminary studies were fruitful, further development of such a theoretical system might be of great benefit in the investigation of the possible effect of such systematic orientational changes on electron transfer rates and ultimately electron transfer distances.

ELECTROCHEMICAL MEDIATORS

Initial electrochemical studies of the behavior of cytochrome c at bare metal electrodes inevitably must have

been discouraging. Here was a molecule capable of donating and accepting electrons, in exactly the same way any typical inorganic transition metal complex would, yet results often categorized the protein as being electroinactive [4,5]. Better preparatory methods of cleaning the working electrode surface prior to its use were investigated, however even the most scrupulous regimen gave at best only very short lived, unstable signals. Typical cyclic voltammograms displayed increasing peak separation and decreasing current amplitudes as the number of scans increased. Bowden, Hawkridge, and Blount suggested, based on these signs of electrochemical irreversibility that the protein was adsorbing onto the gold surface, effectively inhibiting any further electron transfer [5].

While a mediator works (in one manifestation) in the same manner, adsorbing onto and blocking the electrode surface, it does not affect adversely the observed electrochemistry of the redox system under study. Hill *et al.* have demonstrated numerous times throughout a series of papers that the electrochemistry of cytochrome c in the presence of a surface modified gold electrode ranges from quasi-reversible to reversible with no depletion of signal after repeated scans [4,6-10]. The source of the ability of a mediator to facilitate electron transfer is the presence of two significant functionalities, one which binds to the electrode surface, while the other ideally interacts

chemically with the compound of interest. In the case of cytochrome c, linear hydrocarbons containing sulfur substituents that adsorb onto the gold electrode surface and nitrogen substituents that attach to the protein itself thus far have been the most commonly employed mediators [6].

An interesting subclass of surface modifiers that has emerged out of the general study of mediators contain those surface modifiers that permit electron transfer to both negatively and positively charged proteins. The functionality interacting with the protein in such modifiers is more elaborate, containing both acidic and basic ionizable functional groups; 4,4'-bipyridine and (pyridinylmethylene)hydrazinecarbothioamides are the first two examples of such compounds [7]. Both have been shown to facilitate electron transfer to negatively as well as positively charged proteins at pH 7. Such modifiers have also been used in studying a single protein as the overall charge the protein is changed by varying the pH over a wide range.

pH DEPENDENT STATES OF CYTOCHROME c

Many denaturation studies of cytochrome c demonstrate that the protein exhibits biphasic behavior. Consider for example Figure 3, which shows the adsorbance of cytochrome c at 400 nm as a function of time. The implication whenever biphasic behavior is observed is that the transition from

native to fully denatured protein must pass through intermediate conformational states [11]. Indeed this is true of cytochrome c. Theorell and Akesson presented the earliest evidence that cytochrome c exists in more than two states as pH is varied. They denatured the protein by titration both with hydrochloric acid and sodium hydroxide, and concluded from both equivalence points as well as spectrophotometric measurements that ferricytochrome c has five chemically distinct pH dependant states, while ferrocyanochrome c has three [12]. Using resonance Raman spectroscopy Myer et al. detected two additional states for ferricytochrome c [13].

Cytochrome c exhibits biphasic behavior in formal potential with varying temperature and pH. In exploiting this fact Ikeshoji, Taniguchi, and Hawkrige found only four electrochemically distinguishable states of ferricytochrome c, and no transition was observed for ferrocyanochrome c below pH 12 [14]. The pKa's of transition from all three studies appear in Table 1. Figure 4, a plot of formal potential versus pH, shows the four electrochemical states. Ikeshoji et al. concluded that the biphasic behavior of cytochrome c results entirely from its ferric form and that the energetic contribution of ferrocyanochrome c to the overall free energy of electron transfer is small. Type III ferrocyanochrome c has been described as being a very tightly folded conformation that is highly unreactive - resistant to

even strong chemical oxidants and reductants [1].

THESIS PROPOSAL

In order to investigate the behavior of the heterogeneous rate constant of cytochrome c with varying pH, we employed cyclic voltammetry using L-cysteine modified gold electrode. The overall charge of cytochrome c is expected to vary with changing pH. L-cysteine should promote electron transfer with both negatively and positively charged cytochrome c as described above. The heterogeneous rate constants were evaluated from the data using the Nicholson peak splitting method.

EXPERIMENTAL PROTOCOL

Information about the source and purity of each chemical appears in Table 2. All chemicals were used as purchased without further purification, except for monobasic and dibasic sodium phosphate, which were both purified by David Conrad as part of his doctoral thesis [15].

All electrochemical data were obtained using either a BAS 100A Electrochemical Analyzer (Bioanalytical Systems) or a cybernetic potentiostat prototype of the commercial instrument. Respectively, the working, counter and reference electrodes used in all experiments were a gold disc with a radius of 2mm (Bioanalytical Systems), platinum wire, and a silver/silver chloride electrode of conventional design in a salt bridge containing 1 M KCl. The electrochemical cell used was also of the same design which appears in the Conrad thesis. The glass chamber used in the cell was made by the School of Chemical Sciences Glass Shop.

Fresh solutions of aqueous buffer (20mM), modifier (1mM) and supporting electrolyte (100mM) were made prior to each experiment by dissolving the solid in Milli-Q water, and adjusting each solution to the desired pH using small amounts of aqueous hydrochloric acid and sodium hydroxide. Cytochrome solutions were then made by dissolving cytochrome c in a solution containing equal amounts of electrolyte and buffer.

The general procedure for cleaning any part of the cell that would come into contact with the cytochrome solution was to soak it in a solution of 35% $\text{H}_2\text{O}_2/\text{H}_2\text{SO}_4$ (1:9 v/v) for four hours, and then to rinse it with small amounts of Milli-q water. All cell parts except for the working electrode were then sonicated for ten minutes in Milli-Q water, after which they were rinsed with additional Milli-Q water. The working electrode was polished on a Microcloth polishing pad (Buehler Ltd.) using an Ecomet 1 Polisher/Grinder (Buehler Ltd.). The electrode was polished for three five minute periods using diamond paste (Metadi) decreasing in size from 3 to 1 to 0.25 microns in that order. The diamond paste was diluted with Milli-Q water during its use. After polishing, the gold electrode was sonicated for 10 minutes in Milli-Q water and then modified by soaking it in L-cysteine for 5 min.

The modified working electrode was transferred directly to a cell containing blank electrolyte. A background scan

was recorded over several cycles in order to verify the stability of the electrode. Blank electrolyte was then removed from the cell and replaced with cytochrome solution. A series of voltammograms at scan rates ramping up from 50 mV/s to 500 mV/s and then back down again were recorded. Additional cysteine (100 μ l) was added to the cell, and the same series of voltammograms recorded again for the same cytochrome solution. The same series of scan rates was repeated for all integer pH values between 4 and 9.

RESULTS AND DISCUSSION

Cathodic and anodic current amplitudes, peak splitting, and the calculated values for Ψ and heterogeneous rate constant are tabulated in Table 3 as functions of changing pH and scan rate. A typical series of voltammograms is depicted in Figure 5.

Voltammograms recorded at pH 9 often showed no evidence of faradaic activity, and were virtually identical in structure to the background scan. Voltammograms obtained from pH 4 to pH 8 displayed very similar structural features from one voltammogram to the next. One peak in both the forward and reverse scans indicated the presence of one redox couple, while the experimentally obtained heterogeneous rate constants further verified according to the guidelines suggested by Matsuda and Ayabe [3] that cytochrome c, in the presence of phosphate buffer over the potential range of +0.250 to -0.200 V vs. Ag/AgCl at a gold

electrode modified with L-cysteine, displays quasi-reversible behavior. The fact that peak separations increased with increasing scan rate, ranging from approximately 90 mV at a scan rate of 50 mV/s to around 150 mV at a scan rate of 500 mV/s, is another indication of quasi-reversible behavior [16].

The data obtained at any integral pH value we studied suggest the electrochemical reaction of cytochrome c at an L-cysteine modified gold electrode is diffusion controlled, as evidenced by the linearity in plots of peak current as a function of the square root of scan rate however, we noticed additionally that the slope of such plots changed from one pH value to the next, ranging from $1.7 \mu\text{Acm}^{-1/2}\text{s}^{-1/2}$ to $3.4 \mu\text{Acm}^{-1/2}\text{s}^{-1/2}$. As a proper analysis lies beyond the focus of this paper, we suggest here only that this phenomenon may be indicative of changing interfacial character, and defer any further elaboration on the subject of changing diffusivity to a later point in time.

Further evidence of interfacial instability may lie in a general trend we observed, that the initial cytochrome solutions seemed to display a rather short lived stability, as indicated by decreasing current amplitudes. Cyclic voltammograms were recorded in all cases until at least one of the current signals was no longer detectable, at which point additional L-cysteine was added to the cell. While

reminiscent of strong absorption onto the electrode surface, electron transfer was never found to be irreversible. Quite to the contrary, the presence of additional surface modifier seemed not only to rejuvenate the signal stability, but often to greatly improve it as well. At low concentrations of surface modifier, cytochrome c may be capable of displacing surface modifier from the working electrode surface. Irreversible adsorption at bare metal electrodes suggests a very strong affinity towards metals, an attractive force that and might only be counteracted in the presence of sufficiently high concentrations of surface modifier. Hill, Page, and Walton have reported that poly-L-lysine is capable of displacing the surface modifier 1,2-bis(4-pyridyl)ethylene in much the same fashion [9,17].

Figure 6, a plot of redox potential versus pH, shows that over essentially a four order of magnitude change in hydrogen ion concentration, the formal potential of cytochrome c did not vary linearly. This is clearly an exception to the trend reported by Rees, that the redox potential of many proteins depends linearly on the overall charge of the molecule [18], however it also is in good agreement with the findings of Ikeshoji, Taniguchi, and Hawkrige [14]. Additionally it suggests that (type III) ferricytochrome c was indeed the object of this investigation, which is the form of cytochrome c that would

be expected to exist at the initially imposed potential of +0.250 V.

While the conformation of cytochrome c does not change, the charge state most certainly does between pH 4 and 8. The molecule must respond to changing overall surface charge in some other manner, such as translation or rotation. The fact that k^0 exhibited much the same sort of independence to varying pH as did the redox potential, makes translation seem unlikely. Rotation on the other hand would be plausible if the changing charge distributions caused cytochrome c to simply spin about an axis more or less perpendicular to the electrode surface, while always remaining in contact with the electrode surface.

An equally justified explanation, based on the trends of E^0 and k^0 , is that the entire electron transfer process took place far enough under the surface of the system that it was effectively shielded from changing surface conditions. We feel this explanation, while certainly plausible, does not take into account an important aspect of our experimental procedure. Our methodology was to adjust the pH of each cell solution separately, and then once the cell had been assembled, record all data at a single pH value. In each case the cytochrome solution was initially not in contact with the gold electrode surface, however in each case we observed a voltammetric response when the two

components were brought into contact with each other. Electron transfer occurs, with little variation in E^0 and k^0 both in the presence of the changing charge state of cytochrome c and inspite of it.

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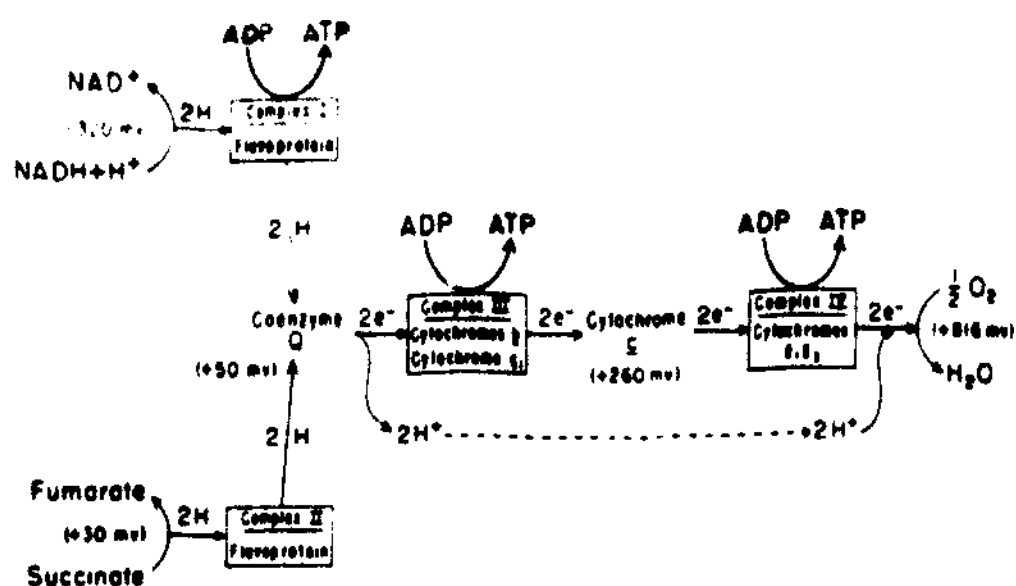


Figure 1. The structure of the mitochondrial respiratory electron transport chain. The indicated measured redox potentials at which electrons are transferred at various points along the chain, occur at pH 7. From: Dickerson R.E.; Timkovich, R. In *The Enzymes*; Boyer, P.D. Ed.; Academic Press: New York, 1976; 3rd Edition, Vol. 11, Chapter 7.

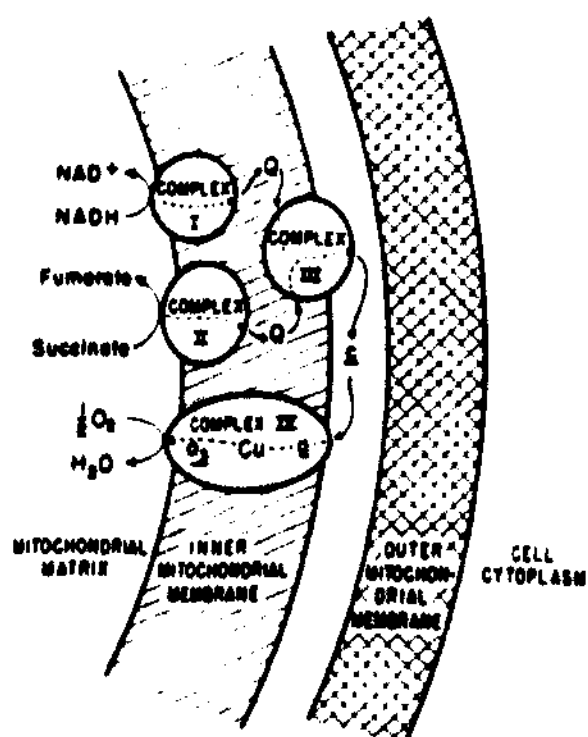


Figure 2. A hypothetical cross section of a mitochondrion, illustrating the role of cytochrome c in mitochondrial electron transport. From: Dickerson R.E.; Timkovich, R. In *The Enzymes*; Boyer, P.D. Ed.; Academic Press: New York, 1976; 3rd Edition, Vol. 11, Chapter 7.

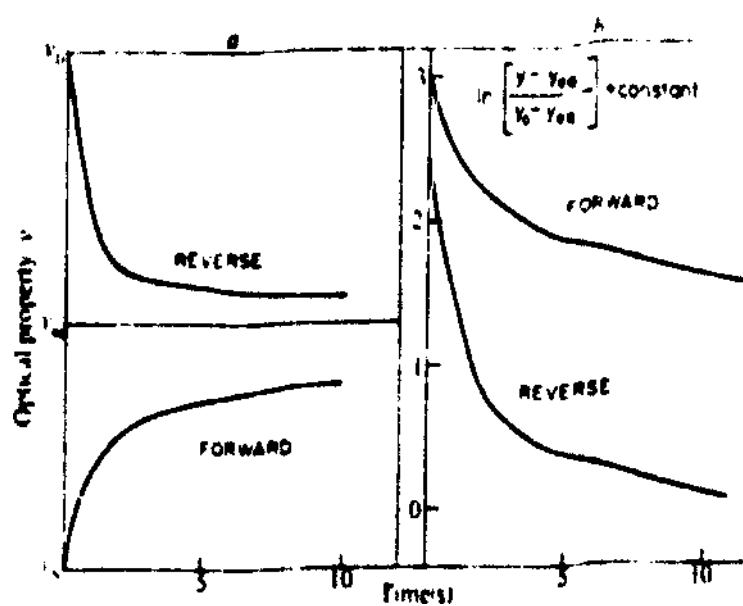


Figure 3. Absorbance of cytochrome c at 400 nm versus time. The presence of two linear regions is indicative of biphasic behavior. From Ikai, A.; Tanford, C. *Nature*; (1971), 100, 230.

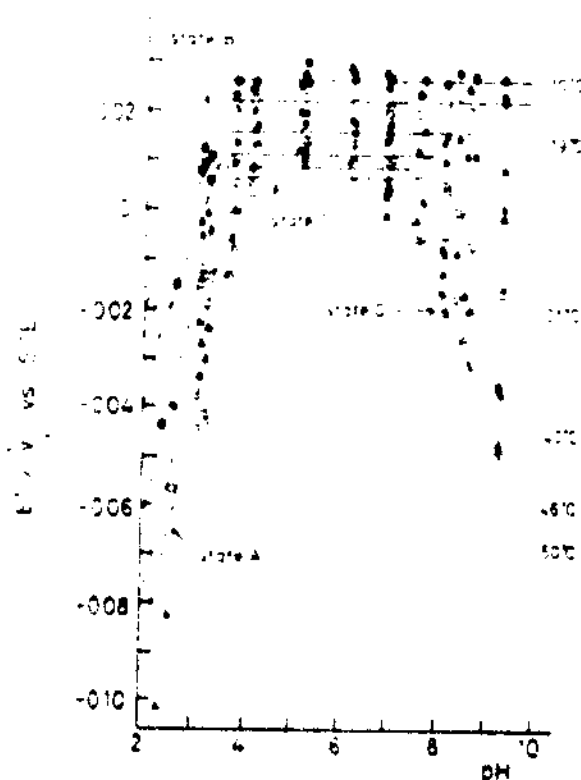


Figure 4. The variation of E^0 with changing pH, illustrating the four electrochemically distinct states of ferricytochrome c. From: Ikeshoji T.; Taniguchi I.; Hawkrige F.M. *J Electroanal. Chem*; (1989), 270, 297-308.

Figure 5. Typical cyclic voltammograms of cytochrome c in phosphate buffer obtained at an L-cysteine modified gold electrode. Batch I, recorded before additional cysteine was added are: i) blank supporting electrolyte - phosphate buffer and scan rates (mV/s) ii) 50 iii) 100 iv) 150 v) 250 vi) 250 vii) 150 viii) 100 xiv) 50. Batch II, recorded after additional cysteine was added are: i) 50 ii) 100 iii) 150 iv) 250 v) 250 vi) 150 vii) 100 viii) 50.

29-JUL-90 16:17:42

SCALE FACTOR = 64

CYCLIC VOLTAMMETRY

500nA

EXP. CONDITIONS:

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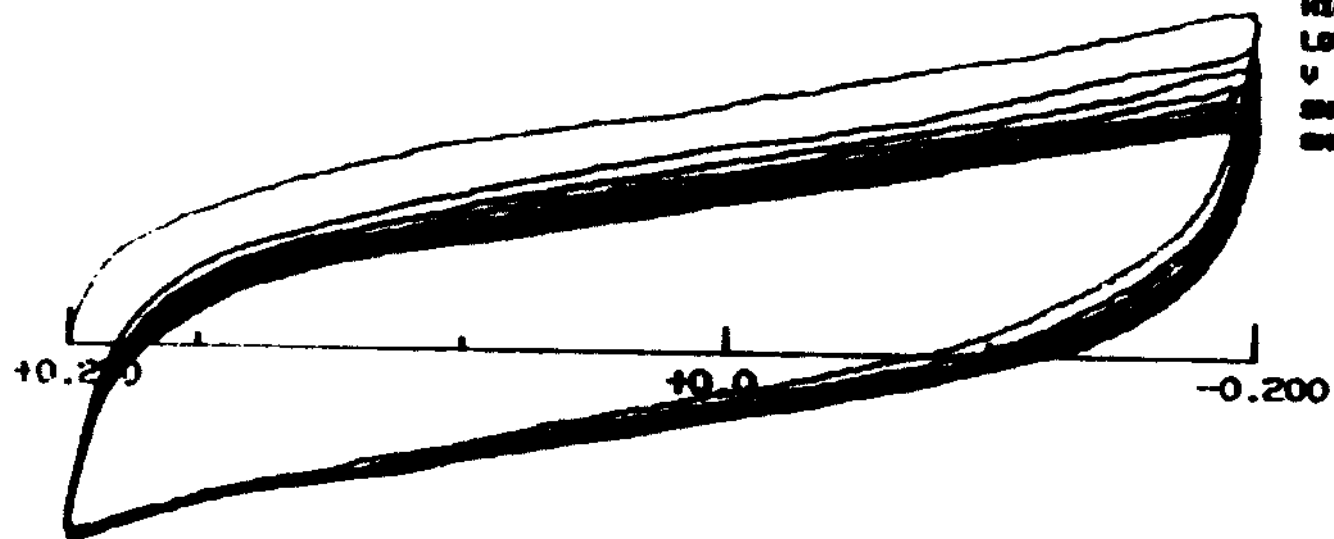
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V (mV/SEC) = 50

SWEEP SEGMENTS = 20

AMPL INT. (mV) = 2



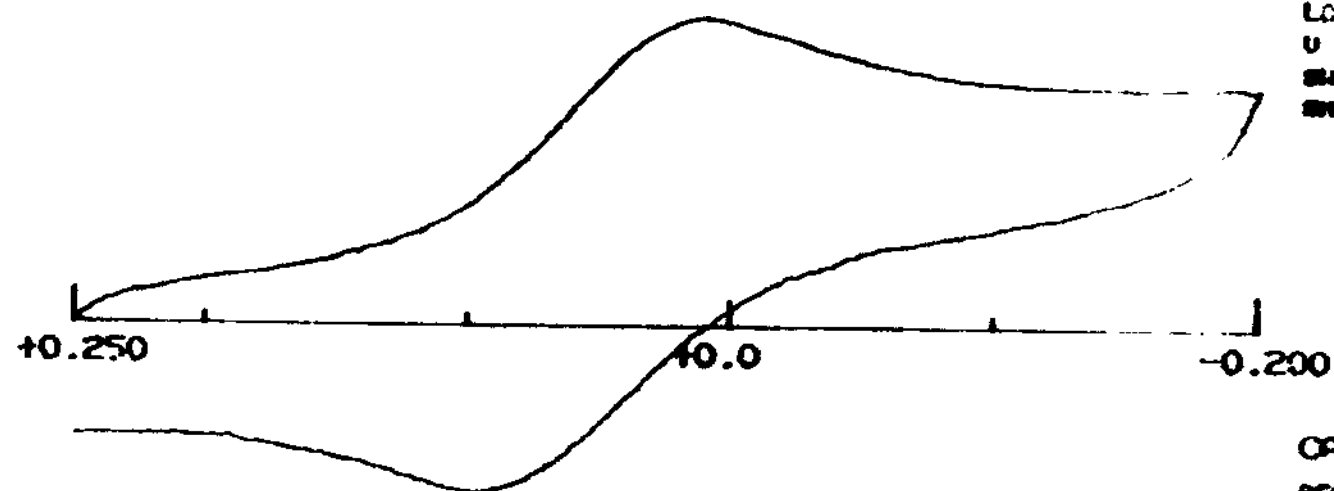
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CYCLIC VOLTAMMETRY

1 μ A



EXP. CONDITIONS:

INIT E(mV) = 250

HIGH E(mV) = 250

LOW E(mV) = -200

U (mV/SEC) = 50

SWEPT SEGMENTS = 2

SWPL INT. (mV) = 1

CATHODIC:

PEAK POTENTIAL (mV) = -110

PEAK CURRENT (A) = 9.7230E -7

ANODIC:

PEAK POTENTIAL (mV) = 110

PEAK CURRENT (A) = 9.0650E -7

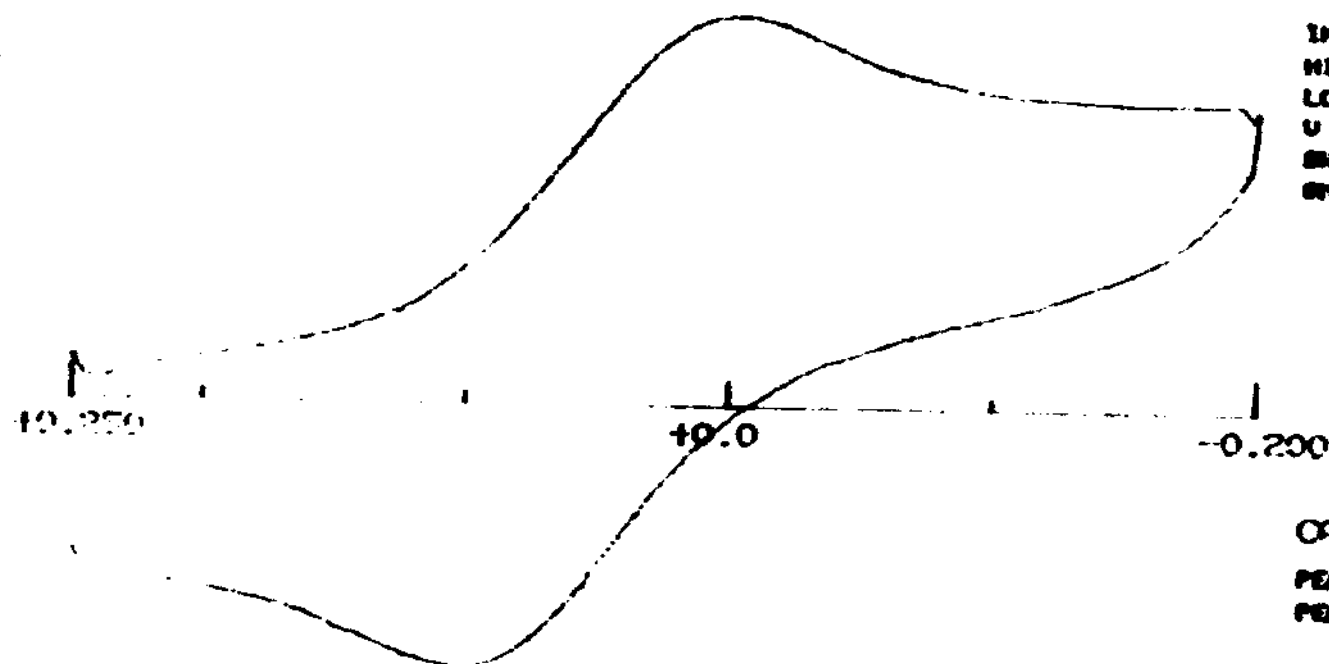
E (VOLT)

29-JUL-90 16:29:19

SCALE FACTOR = 128

CYCLIC VOLTAMMETRY

1.00



EXP. CONDITIONS:

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HIGH E(mV) = 250

LOW E(mV) = -200

U (mA/SEC) = 100

SWEEP SEQUENCE = 2

SWPL INT. (mV) = 1

CATHODIC:

PEAK POTENTIAL (mV) = -3

PEAK CURRENT (A) = 1.887E -6

ANODIC:

PEAK POTENTIAL (mV) = +103

PEAK CURRENT (A) = 1.1794E -6

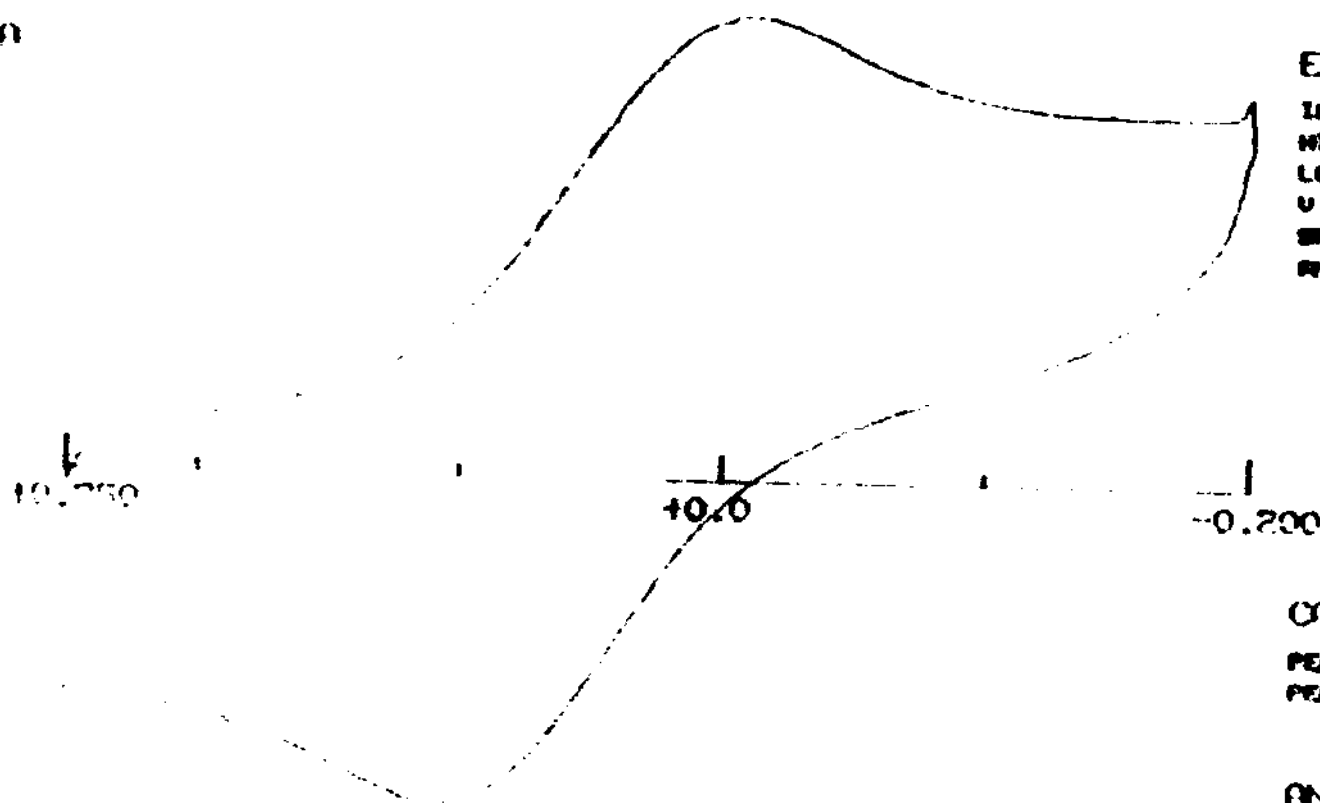
E (VOLT)

29-JUL-90 16:32:13

SCALE FACTOR = 128

CYCLIC VOLTAMMETRY

1.00



EXP. CONDITIONS:

INIT E(mV) = 250

HIGH E(mV) = 250

LOW E(mV) = -200

V (mV/SEC) = 150

SWEEP SEGMENTS = 2

AMPL INT. (mV) = 1

CATHODIC:

PEAK POTENTIAL (mV) = -14

PEAK CURRENT (A) = 1.4780E -6

ANODIC:

PEAK POTENTIAL (mV) = +106

PEAK CURRENT (A) = 1.2889E -6

E (VOLT)

29-JUL-90 16:35:07

SCALE FACTOR = 256

CYCLIC VOLTAMMETRY

256

EXP. CONDITIONS:

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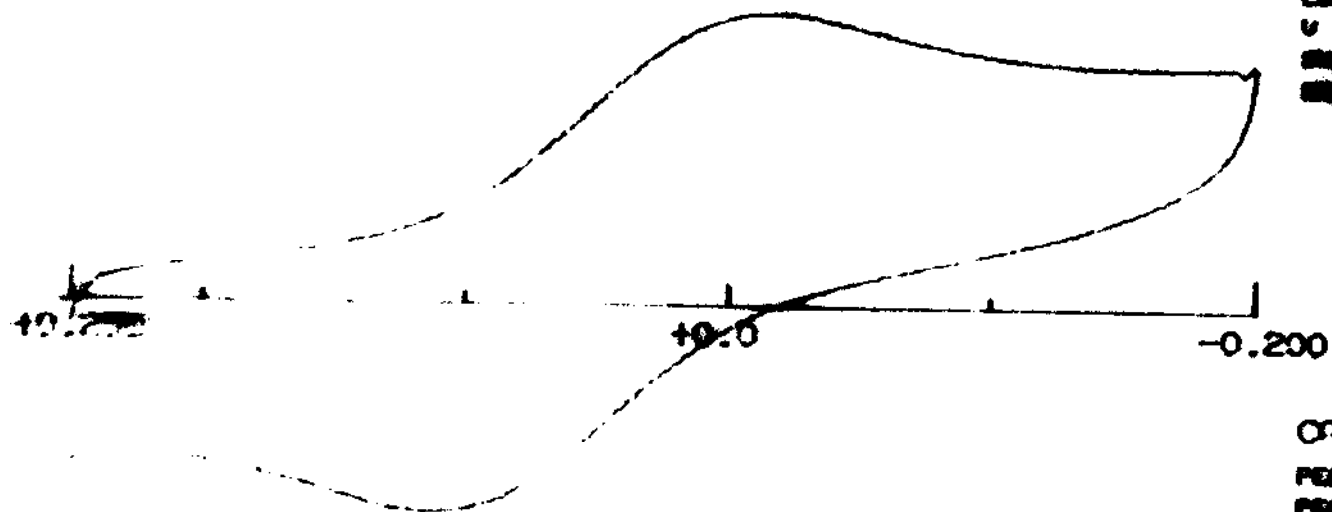
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LOW E(mV) = -200

V (mV/SEC) = 250

SWEEP SEGMENTS = 2

REFL INT. (mV) = 1



CATHODIC:

PEAK POTENTIAL (mV) = -20

PEAK CURRENT (A) = 1.400E -6

ANODIC:

PEAK POTENTIAL (mV) = +115

PEAK CURRENT (A) = 1.400E -6

F. (VOLT)

29-JUL-90 16:44:03

SCALE FACTOR = 256

CYCLIC VOLTAMMETRY

2.5

EXP. CONDITIONS:

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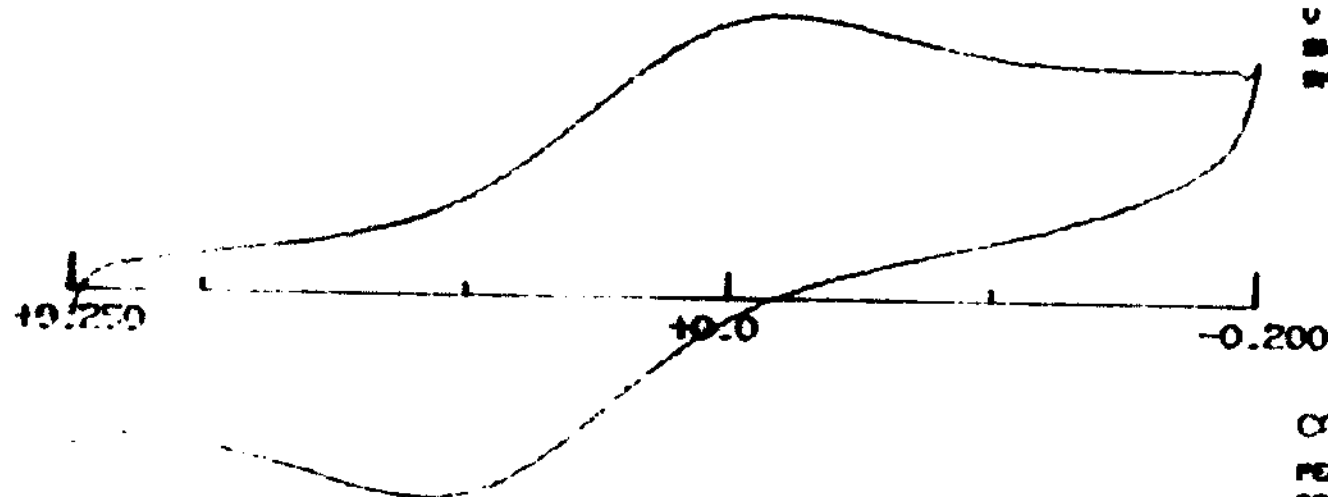
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V (mV/SEC) = 250

SWEEP SEGMENTS = 2

AMPL INT. (mV) = 1



CATHODIC:

PEAK POTENTIAL (mV) = -21

PEAK CURRENT (A) = 1.7979E -6

ANODIC:

PEAK POTENTIAL (mV) = +115

PEAK CURRENT (A) = 1.4249E -6

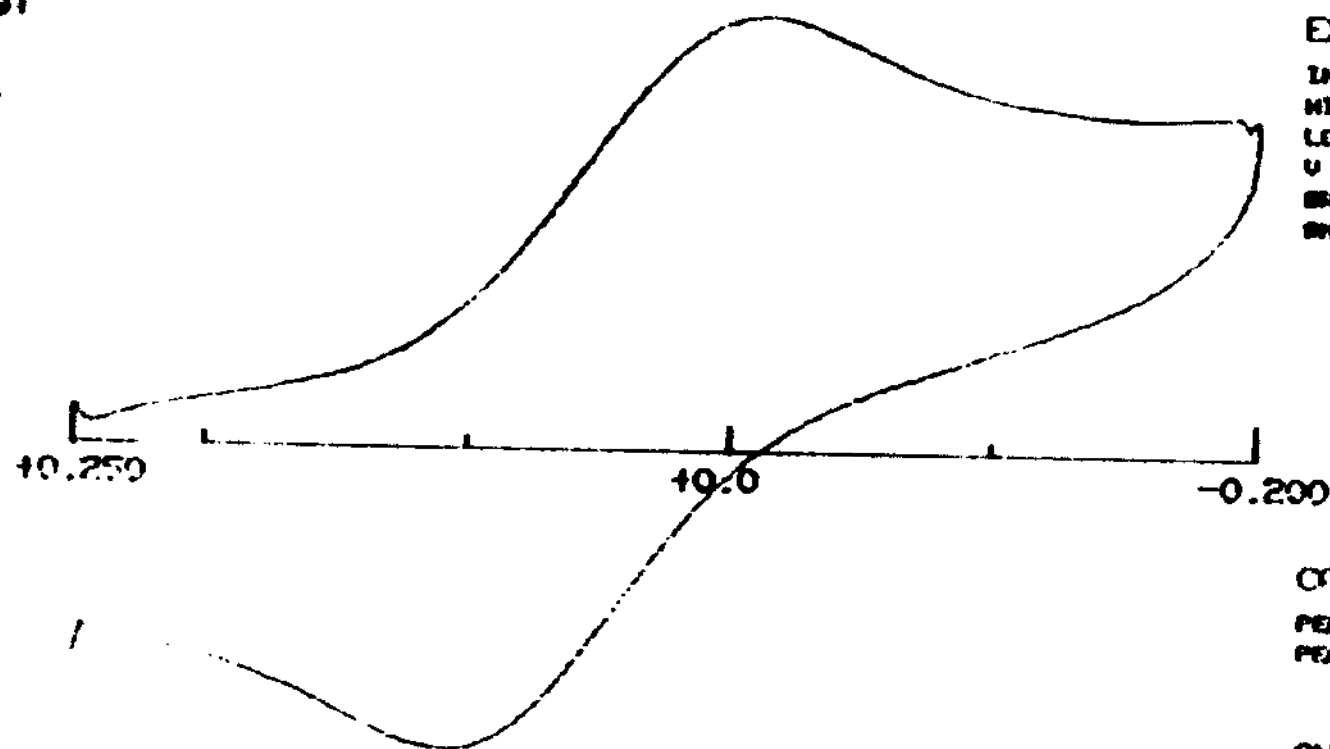
E (VOLT)

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SCALE FACTOR = 120

CYCLIC VOLTAMMETRY

1.00



EXP. CONDITIONS:

INIT E(mV) = 250

HIGH E(mV) = 250

LOW E(mV) = -200

V (mV/SEC) = 150

SWEEP SECTIONS = 2

AMPL INT. (mV) = 1

CATHODIC:

PEAK POTENTIAL (mV) = -12

PEAK CURRENT (A) = 1.8015E -6

ANODIC:

PEAK POTENTIAL (mV) = +107

PEAK CURRENT (A) = 1.2175E -6

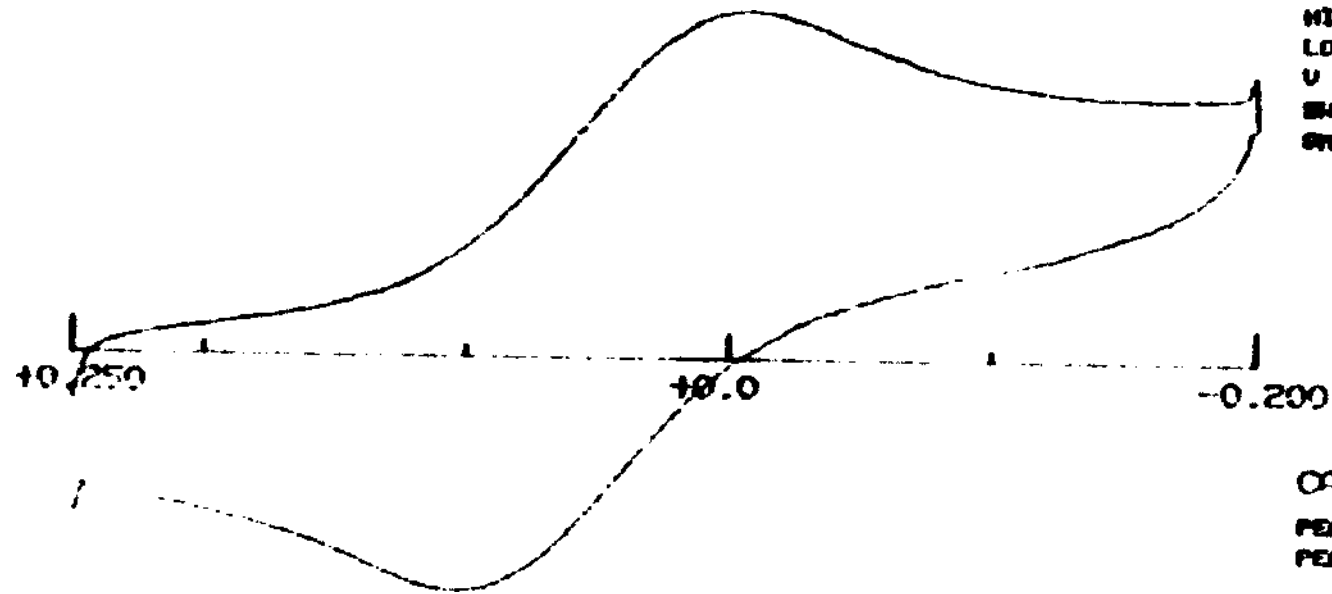
E (VOLT)

29-JUL-90 16:50:01

SCALE FACTOR = 100

CYCLIC VOLTAMMETRY

1 μ A



EXP. CONDITIONS:

INIT E(mV) = 250

HIGH E(mV) = 250

LOW E(mV) = -200

V (mV/SEC) = 100

SWEEP SEQUENCE = 2

SWPL INT. (mV) = 1

CATHODIC:

PEAK POTENTIAL (mV) = -4

PEAK CURRENT (A) = 1.2495E -6

ANODIC:

PEAK POTENTIAL (mV) = +105

PEAK CURRENT (A) = 1.0854E -6

E (VOLT)

29-JUL-90 16:52:52

SCALE FACTOR = 120

CYCLIC VOLTAMMETRY



EXP. CONDITIONS:

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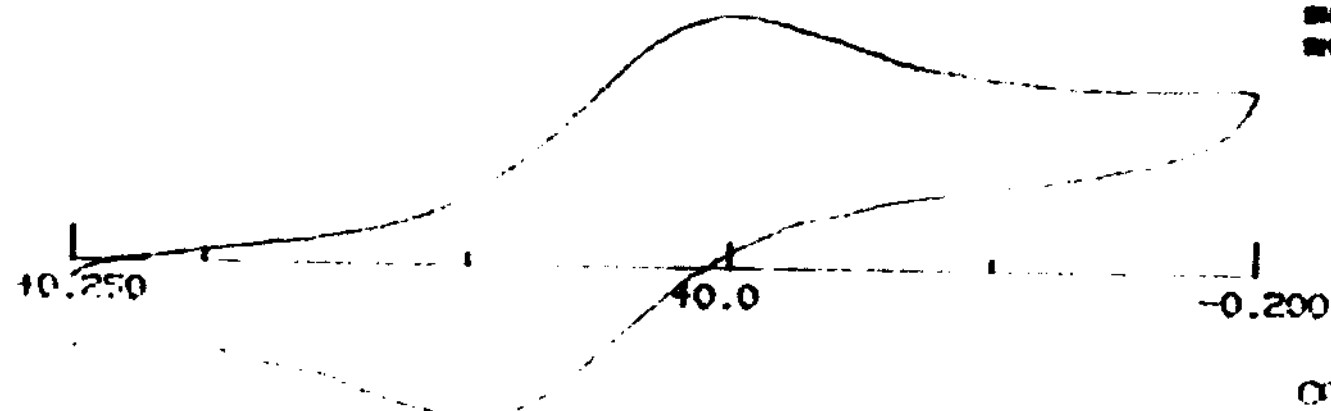
HIGH E(mV) = 250

LOW E(mV) = -250

V (mV/SEC) = 50

SWEEP SEGMENTS = 2

AMPL INT. (mV) = 1



CATHODIC:

PEAK POTENTIAL (mV) = -2

PEAK CURRENT (μA) = 0.50175 -7

ANODIC:

PEAK POTENTIAL (mV) = 499

PEAK CURRENT (μA) = 0.56136 -7

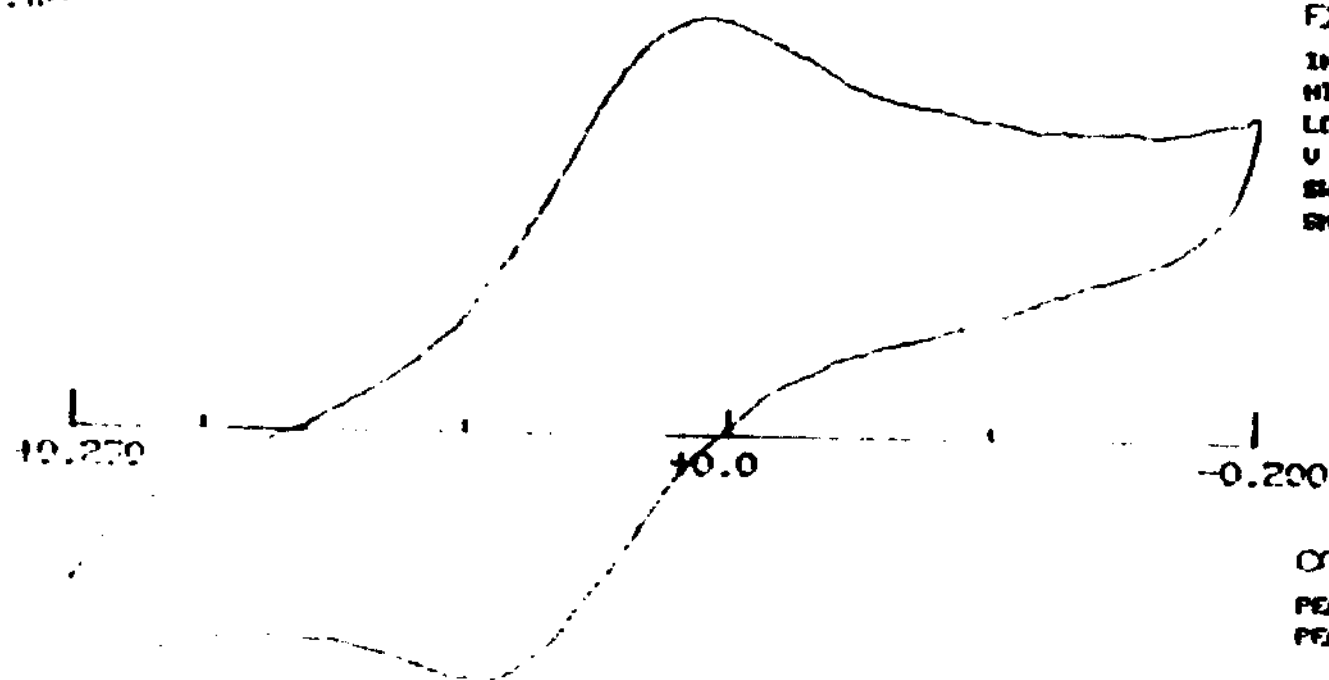
E (VOLT)

29-JUL-90 16:55:47

SCALE FACTOR = 64

CYCLIC VOLTAMMETRY

500nA



EXP. CONDITIONS:

INIT E(mV) = 250

HIGH E(mV) = 250

LOW E(mV) = -200

V (mV/SEC) = 50

SWEPT SEGMENTS = 7

SAMPL INT. (mV) = 1

CATHODIC:

PEAK POTENTIAL (mV) = -118

PEAK CURRENT (nA) = 5.8915E -7

ANODIC:

PEAK POTENTIAL (mV) = 156

PEAK CURRENT (nA) = 5.9997E -7

E (VOLT)

29-JUL-90 16:59:43

SCALE FACTOR = 120

CYCLIC VOLTAMMETRY

1 μ A

EXP. CONDITIONS:

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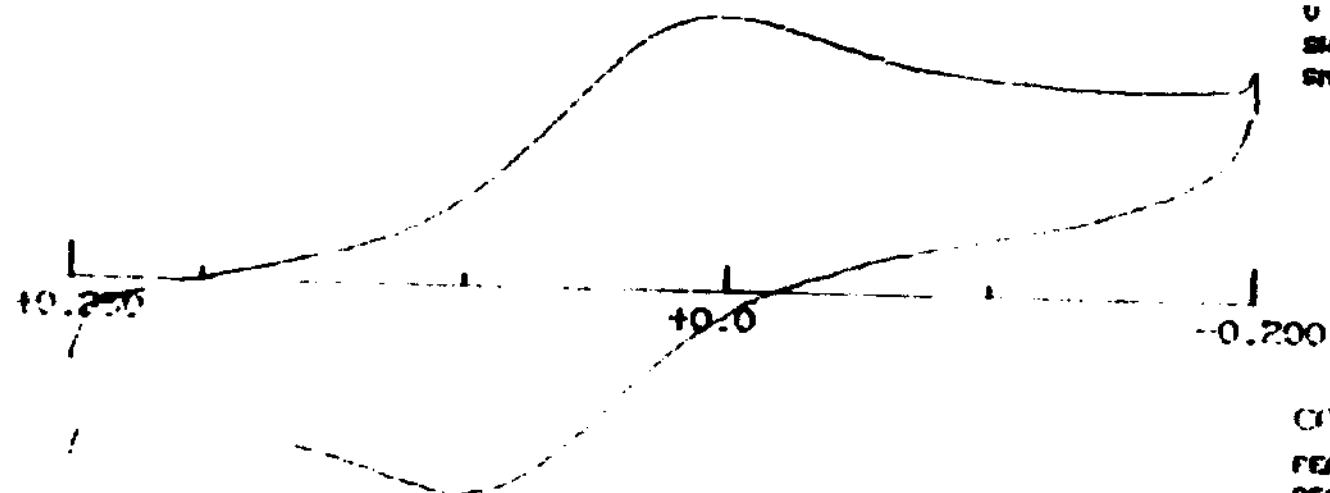
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LOW E(mV) = -200

V (mV/SEC) = 100

SWEEP SEGMENTS = 2

SAMPL INT. (mV) = 1



CATHODIC:

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PEAK CURRENT (A) = 0.3630E -7

ANODIC:

PEAK POTENTIAL (mV) = 100

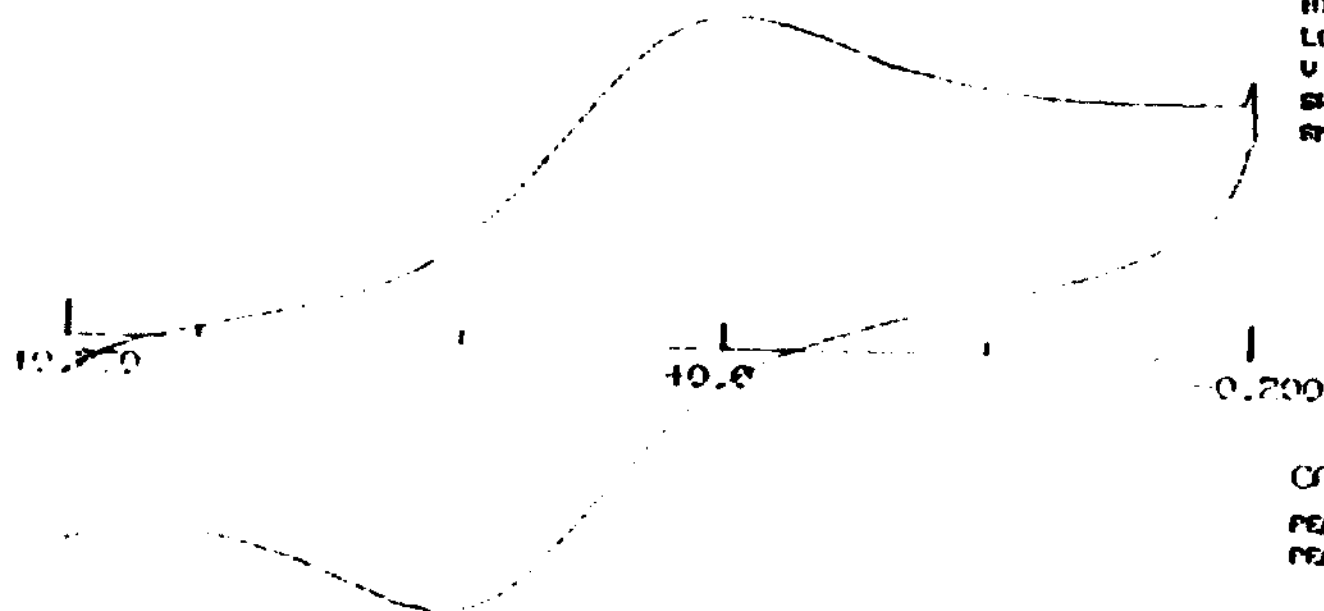
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E (VOLT)

29-JUL-90 17:02:39

SCALE FACTOR = 120

CYCLIC VOLTAMMETRY



EXP. CONDITIONS:

INIT E (mV) = 250

HIGH E (mV) = 250

LOW E (mV) = -200

V (mV/SEC) = 150

SWEEP SEGMENTS = 9

AMPL INT. (mV) = 1

CATHODIC:

PEAK POTENTIAL (mV) = -4

PEAK CURRENT (nA) = 1.027 nA -6

ANODIC:

PEAK POTENTIAL (mV) = +100

PEAK CURRENT (nA) = 9.915 nA +7

E (VOLT)

29-JUL-90 17:06:04

SCALE FACTOR = 120

CYCLIC VOLTAMMETRY



EXP. CONDITIONS:

INIT E(mV) = 250

HIGH E(mV) = 250

LOW E(mV) = -200

V (mV/SEC) = 250

SWEEP SECTIONS = 2

REF. INT. (mV) = 1

10.0

10.0

-0.700

RESULTS:

PEAK POTENTIAL (mV) = -5

PEAK CURRENT (A) = 1.3037E -6

ANODIC:

PEAK POTENTIAL (mV) = 4100

PEAK CURRENT (A) = 1.7209E -6

E (mV)

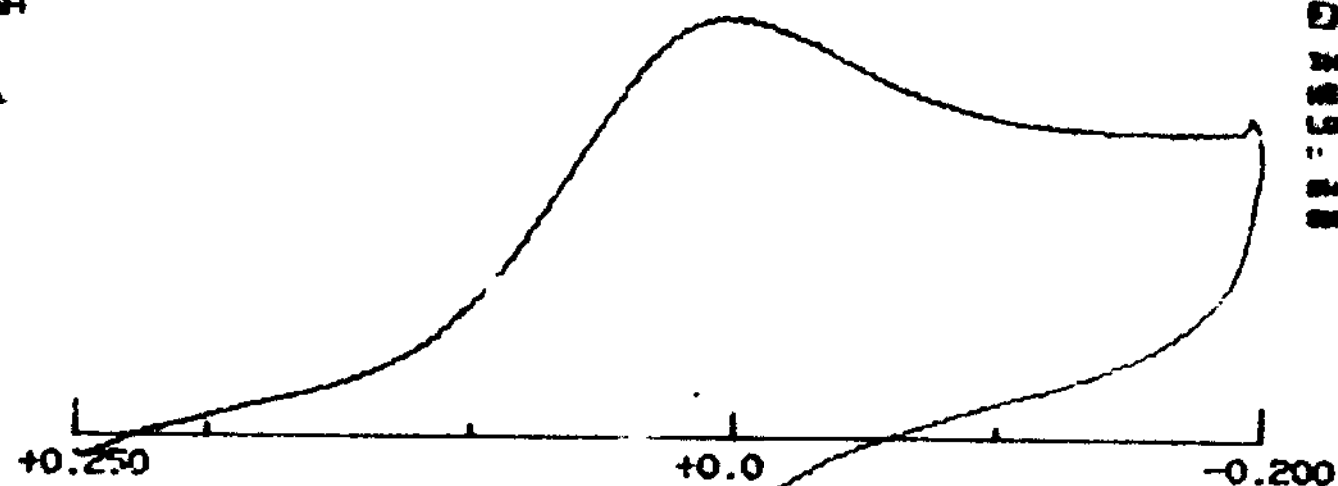
v)

29-JUL-90 17:12.46

SCALE FACTOR = 100

CYCLIC VOLTAMMETRY

1 μ A



EXP. CONDITIONS:

INIT E (mV) = 250

WHEN E (mV) = 250

LEN E (mV) = -200

V (mV/SEC) = 250

SWEEP SEGMENTS = 2

SAMPL INT. (mV) = 1

CATHODIC:

PEAK POTENTIAL (mV) = +3

PEAK CURRENT (A) = 1.4300E -6

ANODIC:

PEAK POTENTIAL (mV) = +96

PEAK CURRENT (A) = 1.3090E -6

E (VOLT)

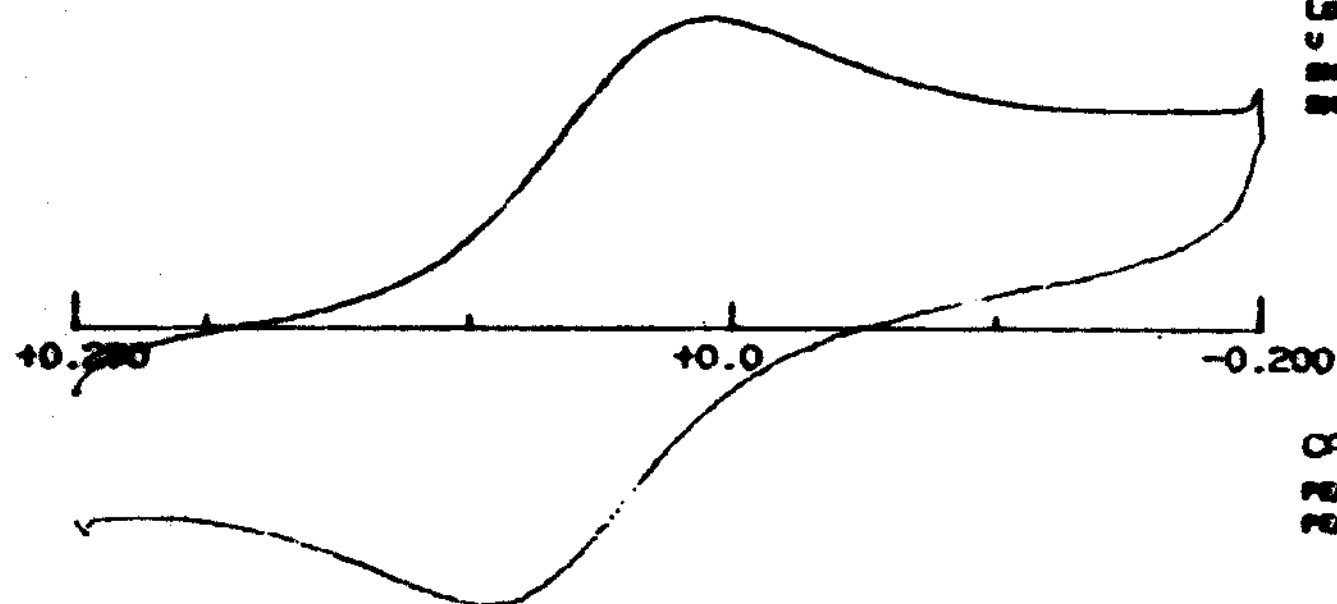
v.)

29-JUL-90 17:15:49

SCALE FACTOR = 120

CYCLIC VOLTAMMETRY

1 μ A



EXP. CONDITIONS:

INIT E (mV) = 250
HIGH E (mV) = 250
LOW E (mV) = -200
V (mV/SEC) = 150
SWEEP SEGMENTS = 2
SPL INT. (mV) = 1

CATHODIC:

PEAK POTENTIAL (mV) = +6
PEAK CURRENT (A) = 1.1337E -6

ANODIC:

PEAK POTENTIAL (mV) = +20
PEAK CURRENT (A) = 1.0470E -6

E (VOLT):

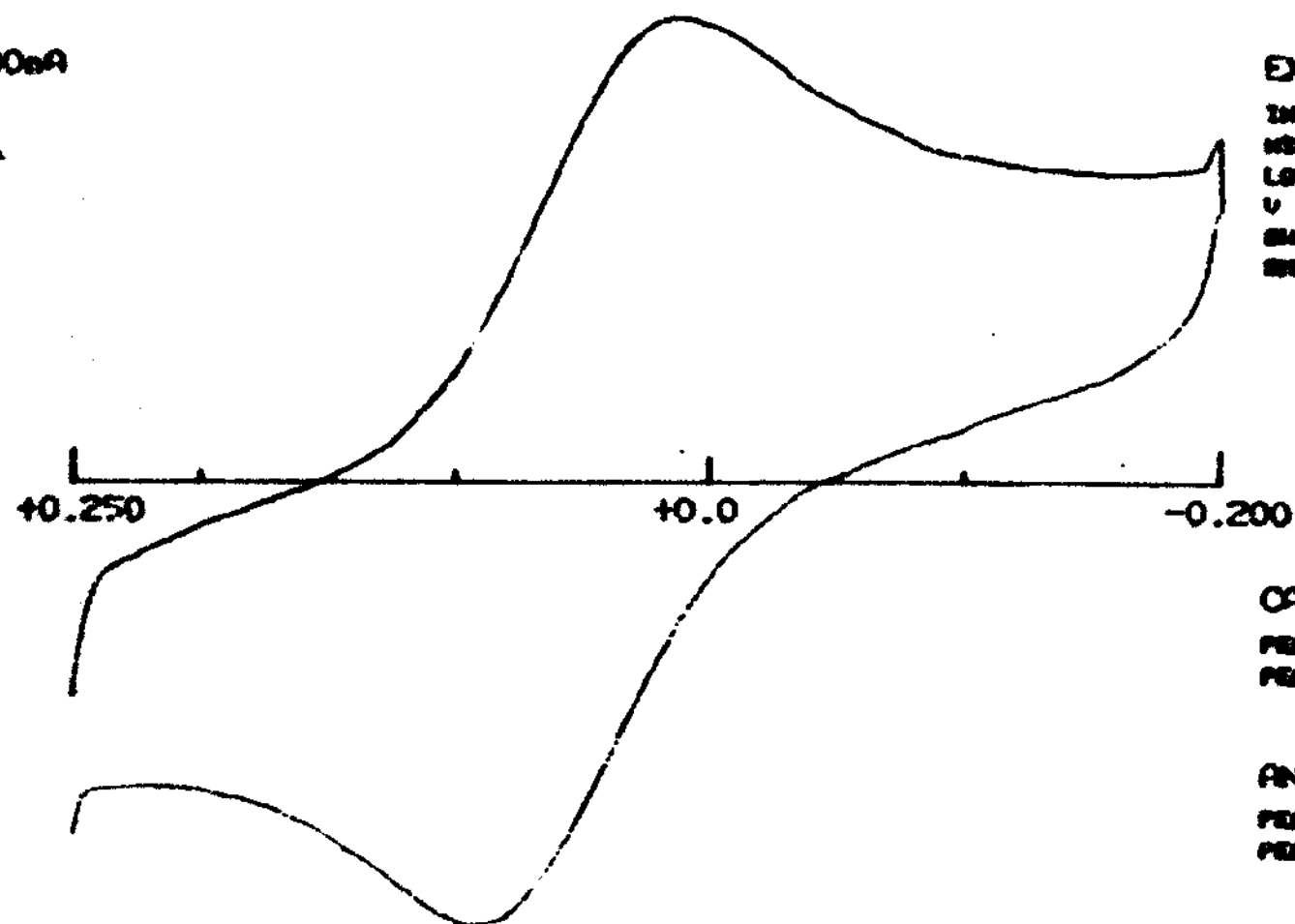
Vol)

29-JUL-90 17:18:40

SCALE FACTOR = 64

CYCLIC VOLTAMMETRY

500nA



EXP. CONDITIONS:

INIT E (mV) = 250
HOLD E (mV) = 250
LOH E (mV) = -200
V (mV/SEC) = 100
SWEEP SEGMENTS = 2
SUPP INT. (mV) = 1

CATHODIC:

PEAK POTENTIAL (mV) = +13
PEAK CURRENT (A) = 1.790E -7

ANODIC:

PEAK POTENTIAL (mV) = +92
PEAK CURRENT (A) = 9.677E -7

E (VOLT)

VOL)

29-JUL-90 17:21:34

SCALE FACTOR = 64

CYCLIC VOLTAMMETRY

500nA

EXP. CONDITIONS:

INIT E (mV) = 250

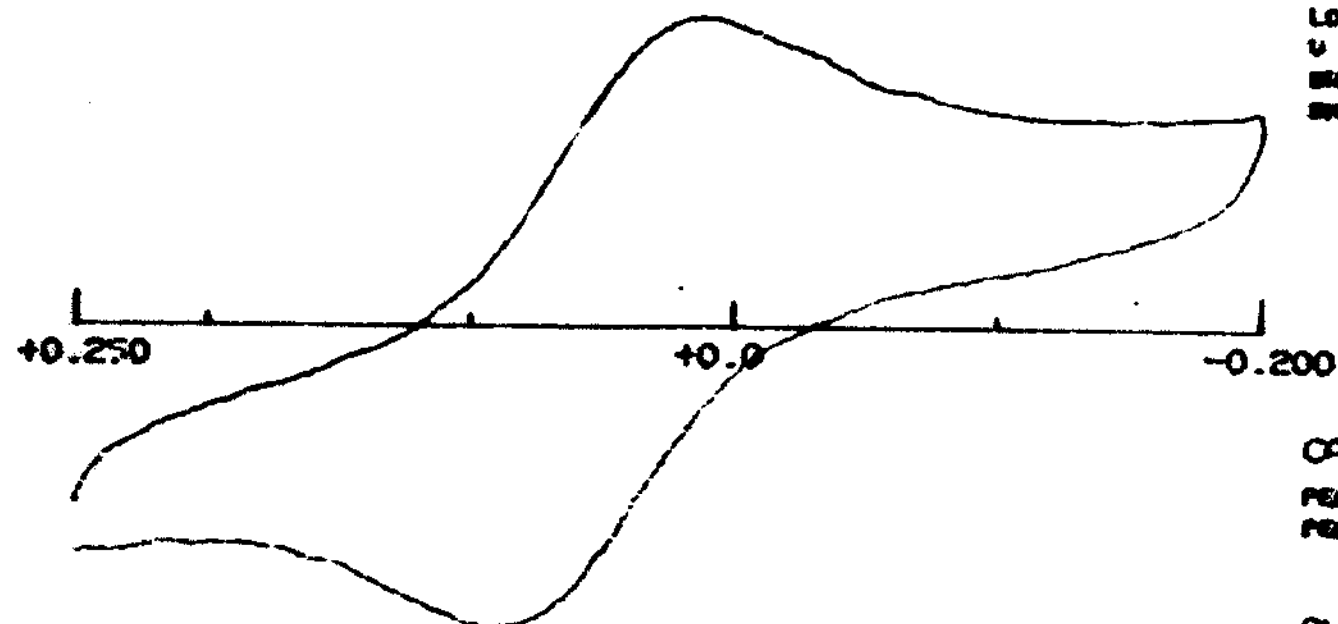
HIGH E (mV) = 250

LOW E (mV) = -200

V (mV/SEC) = 50

SWEEP SEGMENTS = 2

AMPL INT. (mV) = 1



CATHODIC:

PEAK POTENTIAL (mV) = +12

PEAK CURRENT (A) = 5.966E -7

ANODIC:

PEAK POTENTIAL (mV) = +40

PEAK CURRENT (A) = 6.7434E -7

E (V) T)

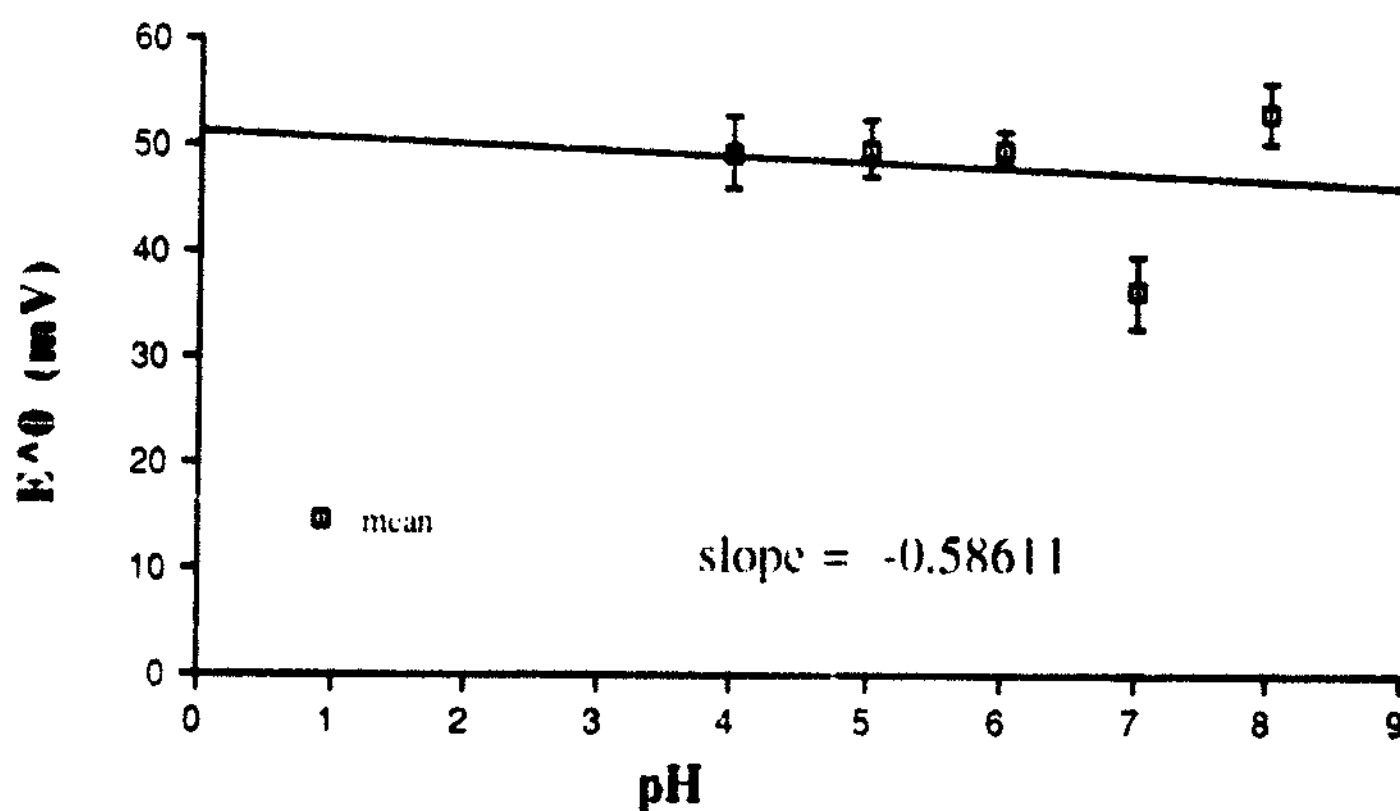


Figure 6. Experimentally obtained plots of formal potential versus pH illustrating the independence of E^0 between pH 4 and pH 8.

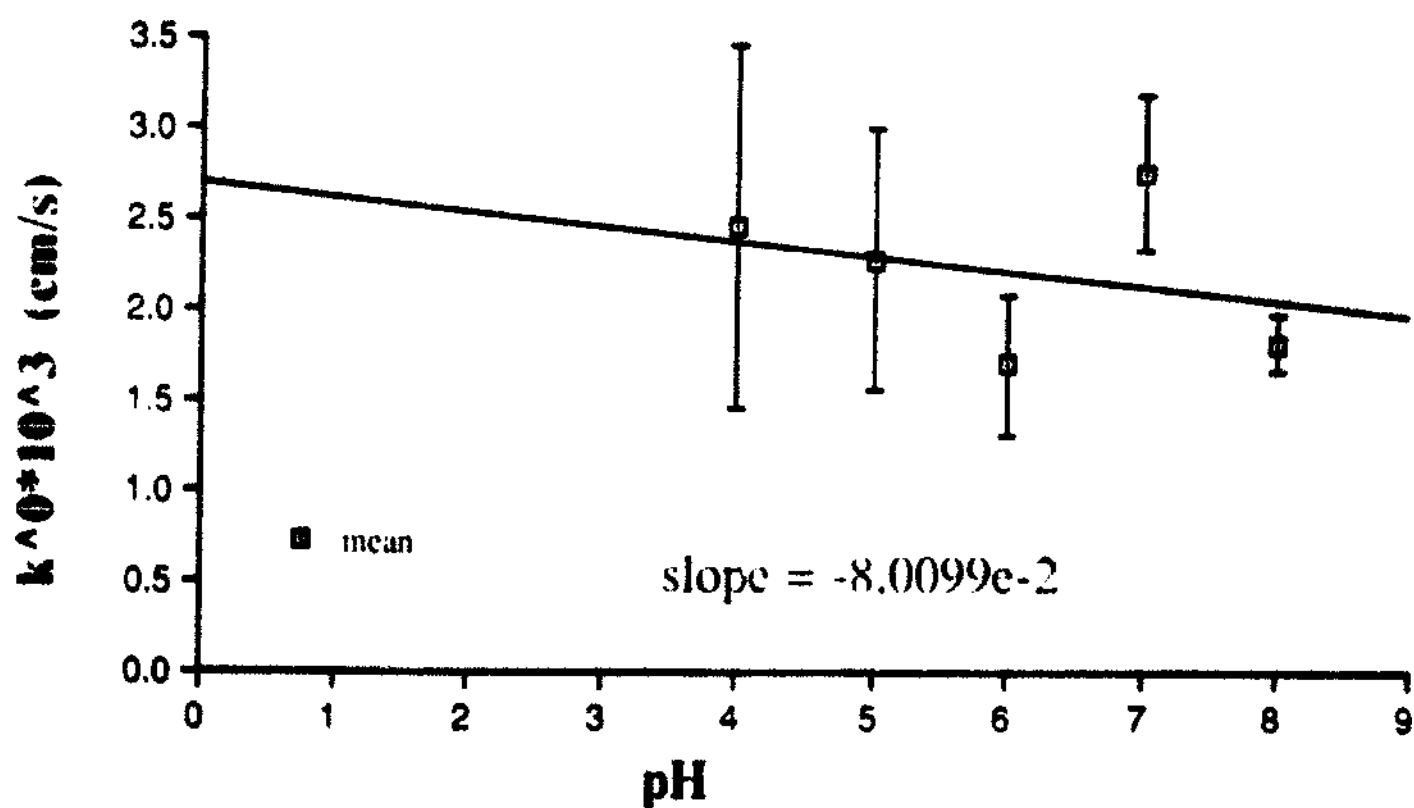


Figure 7. Experimentally obtained plots of heterogeneous rate constant versus pH illustrating the independance of k^0 between pH 4 and pH 8.

i)	States	oxidized, reduced, and reduced + protonated
ii)	pH	1.5, 4.5, 10.5
iii)	States	oxidized, reduced, and reduced + protonated
iv)	pH	1.5, 4.5, 10.5

Table 1. Comparison of the pKa's defining the distinct states of cytochrome c from references: i) [1] ii) [13] iii) [14]. From: Ikeshoji T.; Taniguchi I.; Hawkrige F.M. *J Electroanal. Chem*; (1989), 270, 297-308.

CHEMICAL	SOURCE	PURITY
L-Cysteine hydrochloride, anhydrous, $C_3H_7NO_2S \text{ HCl}$	Fluka Chemical Corp.	>99%
Horse Heart Cytochrome <u>c</u> (Type VI)	Sigma Chemical Co.	95-100%
Hydrochloric acid, HCl	Fisher Scientific Ltd.	reagent
Hydrogen peroxide, H_2O_2	Fisher Scientific Ltd	reagent
Sodium phosphate, dibasic, anhydrous, Na_2HPO_4	J. T. Baker, Fischer	reagent
Sodium phosphate, monobasic, monohydrate, $NaH_2PO_4 \cdot H_2O$	J. T. Baker, Fischer	reagent

Table 2. Source and purity of chemicals used.

PH	α (s)	DC (A)	DC (A)	IC (A)	P ₁	$\alpha \cdot 10^{-3}$
8.000	0.050					
	0.100	0.925	0.731	51.500	0.481	1.844
	0.150	1.170	1.080	52.000	0.450	2.113
	0.250	1.430	1.400	55.500	0.300	1.816
	0.400	1.770	1.950	53.000	0.214	1.833
	0.500	2.030	2.420	53.000	0.221	1.898
	0.750	1.480	1.510	51.500	0.311	1.881
	0.150	1.210	1.770	52.500	0.388	1.826
	0.100	1.040	0.875	52.500	0.441	1.888
	0.050	0.881	0.871	54.500	0.556	1.505
7.000	0.050	0.794	0.730	37.000	0.757	2.052
	0.100	0.888	1.030	36.000	0.757	2.051
	0.150	1.050	1.220	36.000	0.600	2.016
	0.250	1.250	1.350	32.500	0.481	2.016
	0.500	1.070	1.320	32.000	0.275	2.358
	0.750	1.11	1.380	33.000	0.518	3.127
	0.150	1.120	1.240	38.000	0.312	3.338
	0.100	1.030	1.040	17.500	0.783	2.998
	0.050	0.823	0.730	42.500	0.838	2.270
6.000	0.050	0.628	0.581	43.500	0.481	1.334
	0.100	0.785	0.888	48.000	0.634	2.427
	0.150	0.841	1.050	45.000	0.450	2.113
	0.250	1.070	1.250	47.000	0.285	1.785
	0.500	1.230	1.640	44.500	0.173	1.480
	0.750	1.120	1.230	45.500	0.300	1.816
	0.150	0.851	0.954	47.000	0.318	1.483
	0.100	0.888	0.873	45.500	0.441	1.888
	0.050	0.888	0.881	48.500	0.441	1.84
5.000	0.050	0.888	0.445	58.000	0.888	2.384
	0.100	1.280	1.370	53.000	0.838	3.584
	0.150	1.480	1.710	52.500	0.734	3.447
	0.250	1.800	2.080	51.500	0.528	3.203
	0.500	2.300	2.830	48.500	0.347	2.888
	0.750	2.480	2.880	48.500	0.280	2.181
	0.150	1.680	2.020	47.500	0.481	2.488
	0.100	1.360	1.680	47.500	0.604	2.388
	0.050	1.180	1.330	47.000	0.570	2.183
	0.050	0.885	1.050	48.800	0.852	1.785
4.000	0.050	1.040	0.879	52.000	0.483	1.334
	0.100	1.180	1.510	47.500	0.877	3.744
	0.150	1.320	1.740	48.500	0.558	2.808
	0.250	1.480	1.820	47.500	0.405	2.484
	0.500	1.780	2.820	44.500	0.248	2.108
	0.750	2.220	2.030	42.000	0.214	1.833
	0.150	1.380	1.600	43.000	0.285	1.726
	0.100	1.150	1.310	47.800	0.431	2.024
	0.050	1.130	1.140	49.000	0.483	1.887
	0.050	0.888	0.880	48.800	0.422	1.148
3.000	0.050	0.910	0.872	54.000	0.808	2.182
	0.100	1.140	1.830	55.000	0.483	1.887
	0.150	1.280	1.480	48.000	0.387	1.721
	0.250	1.480	1.810	47.500	0.180	1.886
	0.500	1.820	2.530	44.500	0.211	1.608
	0.750	1.780	2.830	48.500	0.205	1.753
	0.150	1.420	1.800	47.000	0.278	1.688
	0.100	1.220	1.800	47.800	0.378	1.788
	0.050	1.080	1.250	50.500	0.488	1.783
	0.050	0.888	0.885	48.800	0.582	1.505
2.000	0.050	0.880	0.583	53.000	0.802	2.444
	0.100	0.878	0.838	53.000	0.483	1.887
	0.150	0.882	1.030	52.000	0.431	2.124
	0.250	1.230	1.380	51.500	0.482	2.558
	0.500	1.878	2.210	48.000	0.471	4.038
	0.750	1.318	1.440	48.500	0.680	4.182
	0.150	1.888	1.100	48.500	0.838	3.832
	0.100	0.888	0.878	52.500	1.81	4.523
	0.050	0.878	0.588	51.000	1.250	3.188

Table 3. Experimentally obtained data, illustrating how E^0 and k^0 vary with changing pH.